

## Ultrastructure of Spermatozoa in *Leptopilina* (Hymenoptera: Cynipoidea: Eucoilidae)

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*Abstract.*—The mature spermatozoa of the eucoilid wasp, *Leptopilina heterotoma* (Thomson), is characterised by a solid corkscrew-shaped nucleus with a posteriorly directed flange that is unique, not only among other reported Hymenoptera, but among all insect sperm previously investigated. *Leptopilina* spermatozoa are further characterised by possession a complex acrosomal structure, asymmetric mitochondrial derivatives and a centriolar adjunct interposed between the smaller mitochondrial derivative and the nucleus. Because of their uniqueness, *Leptopilina* sperm offer little insight into the relationship between the eucoilids and other members of the Cynipoidea, or the relationships of the Cynipoidea to the rest of the Hymenoptera.

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The Hymenoptera is one of the largest orders of insects, but despite this there have been remarkably few studies of hymenopteran sperm ultrastructure, and particularly among the parasitic taxa considering their diversity and species richness (Baccetti 1972; Jamieson 1987; Newman and Quicke, in press). The sperm of some social taxa such as ants and bees (Hoage and Kessel 1968; Lensky et al. 1979; Thompson and Blum 1967; Wheeler et al. 1990; Dallai and Afzelius 1990), and a few Chalcidoidea among the 'Parasitica' (Wilkes and Lee 1965; Lingmei and Dunsu 1987) are relatively well described, but there are still a number of superfamilies and many families for which there is very little spermatological information. Sperm structure in the superfamily Cynipoidea, for example, is known only through one investigation of a member of the family Figitidae (Quicke et al. 1992). The relationship of the Cynipoidea to the other apocritan (wasp-waisted) Hymenoptera is not well understood. They have been considered as being related to the Chalcidoidea

(Königsmann 1977), but also to Diapriidae (Proctotrupeoidea *sensu lato*) (Rasnitsyn 1988; Ronquist 1994, 1995) and to Roproniidae + Vanhorniidae (Proctotrupeoidea *sensu stricto*) (Dowton and Austin 1994). Greater knowledge of the sperm structure of other cynipoids may therefore shed new light on the relationships of this superfamily to the other Hymenoptera.

The present paper deals with the sperm of another member of the superfamily Cynipoidea, *Leptopilina heterotoma* (Thomson) (Quicke 1997), a koinobiont endoparasitoid of *Drosophila* larvae. This is the first member of its family, the Eucoilidae, whose sperm morphology has been investigated. It has a solid-corkscrew nucleus, that is not only different to that of the only other cynipoid so far described, but is unlike any other Hymenopteran sperm previously reported.

### MATERIAL AND METHODS

Testes and vas deferens from freshly eclosed adult males of *Leptopilina heterotoma*, kept in culture on their host (*Drosophi-*

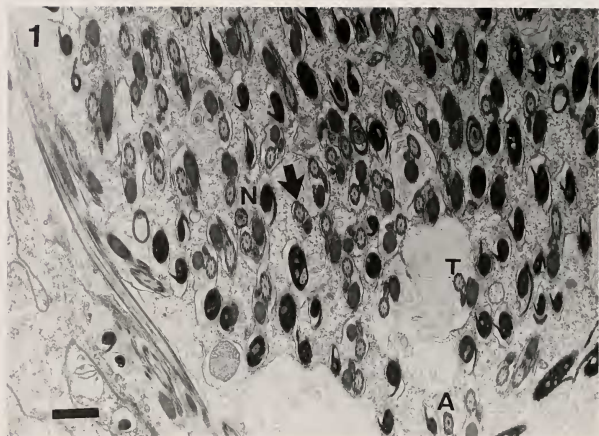


Fig. 1. Low power view of sectioned vas deferens of *Leptopilina heterotoma* with sections through acrosome (A), nucleus (N) and tail piece (T) including some with only one mitochondrial derivative (arrowed) (scale bar = 1  $\mu$ m).

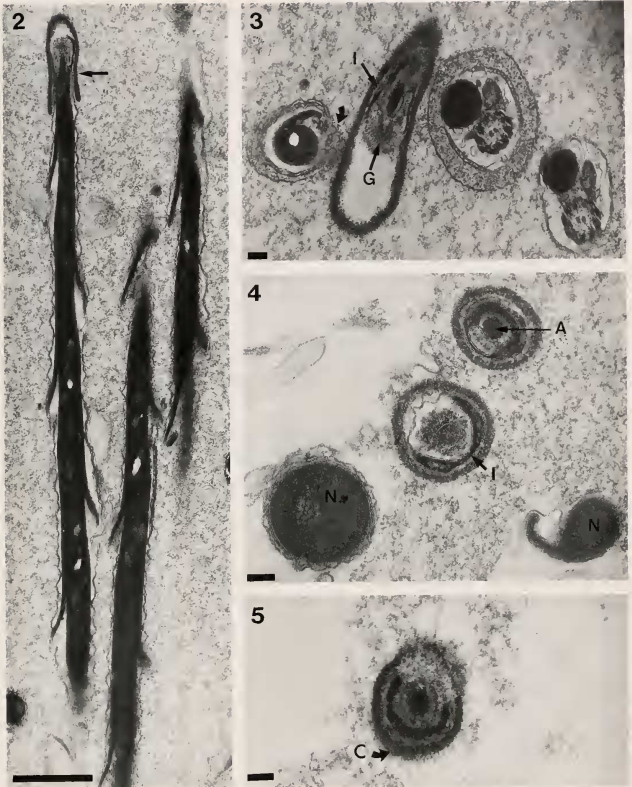
*la melanogaster*) were studied. Preparation was based on the protocol used by Newman and Quicke (1998). The tissues were dissected under 2% glutaraldehyde in phosphate buffered saline (0.1 M; pH 7.2), fixed for two hours, then transferred to 2% osmium tetroxide in cacodylate buffer (0.1 M; pH 7.2) for a further 2 hr fixation. After two buffer washes, tissue was dehydrated to 50% ethanol and then contrasted with a saturated solution of uranyl acetate in 50% ethanol prior to complete dehydration, embedding in Epon resin and polymerisation overnight. Large silver sections were picked up on high resolution grids and contrasted with uranyl acetate and lead citrate.

## RESULTS

The mature sperm of *Leptopilina heterotoma* exhibit many of the structures described for other parasitic wasps, e.g. cristate mitochondrial derivatives, deltoid bodies and an axoneme with 9+9+2 arrangement of microtubules (Fig. 1). However, they also have several features different from previously reported examples. The acrosome is a much more complicated structure than any yet described, forming part of an acrosomal complex (Fig. 2 arrow). Posteriorly the acrosomal rod (perforatorium) lies to one side of the nucleus and is not held tightly there. The anterior portion of the acrosomal rod protrudes

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Figs. 2-5. Features of *Leptopilina heterotoma* sperm: 2, longitudinal section with acrosomal complex surrounded by extracellular cap (arrowed) and showing alternating protuberances from the nucleus which are the result of sectioning through the spiral ridge (scale bar = 1  $\mu$ m); 3, high power oblique section through



acrosomal complex illustrating the extracellular cap, acrosomal rod, inner sheath (I), granular mass (G), granular extracellular cap surrounding anterior part of nucleus (curved arrow, left hand section) [note also more posterior section to right of middle which still has cytoplasm indicative of immature sperm] (scale bar = 100  $\mu$ m); 4, transverse sections through nucleus (N) showing (lower right section) comma-shaped profile characteristic of anterior and medial part, and (left section) round profile towards posterior; acrosomal complex (middle two sections), showing asymmetric electron dense inner sheath of acrosome (I) and acrosomal rod (A) (scale bar = 100  $\mu$ m); 5, transverse section through acrosomal complex at level of acrosomal rod, illustrating that the inner sheath does not completely enclose the granular material of the sub-acrosomal space, and showing denser structure of extracellular cap (C) adjacent to inner sheath (scale bar = 100  $\mu$ m).

into a mass of fine fibrous material which partially fills the sub-acrosomal space (Figs. 2-5). The true acrosome, that is the portion derived from the acrosomal vesicle, has a conical shape. This forms a membrane-bound inner sheath below the true cell plasma membrane (Fig. 3). The acrosomal contents are asymmetrically distributed around the fibrous material. Thus, posteriorly, in section, (Fig. 4, *upper profile*) there is almost a complete ring of material, whereas more anteriorly (Fig. 4, *middle profile*), on one side of the acrosomal complex the acrosomal contents are absent and only the membrane is found. Surrounding this acrosomal complex is an extracellular cap (Figs. 2 and 5). In longitudinal section this cap covers, and completely encloses, the anterior of the nucleus (Figs. 2, 3 *curved arrow*).

It is in the structure of the nucleus that the main difference between the sperm of *Leptopilina* and those of other hymenoptera studied to date is found. In most transverse nuclear sections, there is a protuberance, giving the nucleus a comma-shaped profile (Figs. 1, 3, 4). In longitudinal section, these protuberances alternate along either side of the nucleus for most of its length (Fig. 2). Posteriorly the nucleus abuts the axoneme at the area of the basal body (Fig. 6), where the nucleus overlaps the larger of two mitochondrial derivatives for a short distance (Fig. 6 *open arrow*). In transverse section (Fig. 7, *arrow*) an electron dense line is found that may be a continuation of a similar structure found at the interface of the axoneme and the nucleus. Next to the axoneme, and also abutting the nucleus, is a large electron-dense centriolar adjunct. (Fig. 8, *open arrow*). Posteriorly, this centriolar adjunct contacts the smaller of the two mitochon-

drial derivatives (Fig. 9). The positioning of these structures is such that at the region of overlap it is possible to obtain oblique sections with centriolar adjunct, mitochondrial derivative and nucleus all present (Fig. 10). Closely apposed to the mitochondrial derivatives are the two deltooid bodies (also called triangular rods; Lensky et al. 1979). Although membranous, their derivation is not clear, but they are probably associated with the mitochondrial derivatives because they are also of different sizes (Fig. 11, *arrows*). This offsetting of the mitochondrial derivatives probably results in the tail piece of the wasp spermatozoon only containing a single (small) mitochondrial derivative.

Observations of living sperm expressed from the vas deferens into saline showed ( $\times 400$ ; phase contrast) that the nucleus is a straight, rigid-appearing structure which appears to rotate around its long axis as because waves that we believe correspond to the nuclear ridges described here can be seen passing posteriorly along them.

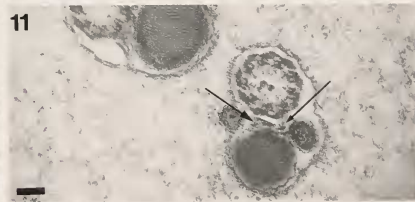
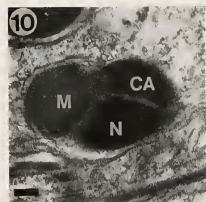
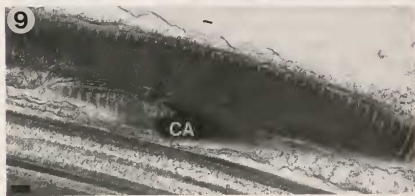
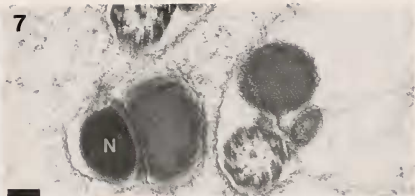
## DISCUSSION

The most probable interpretation of the nuclear structure, as derived from longitudinal and transverse sections, is a cylinder with a posteriorly-directed ridge spiralling down along its surface. This therefore resembles a solid-cored cork-screw. Observations of living sperm indicate that the profile of the sperm head does follow the nuclear cork-screw, and that this shape may serve some fluid dynamic function, perhaps inducing the observed rotatory motion. The spiral ridge may therefore have significance for the fertilisation process.

Spirally formed sperm are known in two other groups of Hymenoptera, an un-

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Figs. 6-11. Features of *Leptopilina heterotoma* sperm: 6, large mitochondrial derivative (open arrow) overlaps the nucleus (N) which abuts the axoneme at the level of the basal body (B) (scale bar = 100  $\mu$ m); 7, transverse section showing overlap of large mitochondrial derivative and nucleus (N), with a electron dense



area separating the two (scale bar = 100  $\mu$ m); 8, centriolar adjunct (open arrow) abutting nucleus (N) anteriorly (scale bar = 100  $\mu$ m); 9, posterior portion of the centriolar adjunct (CA) abuts the smaller mitochondrial derivative (scale bar = 100  $\mu$ m); 10, showing close proximity of the nucleus (N) mitochondrial derivative (M) and centriolar adjunct (CA) which means that in oblique sections it is possible to have all three in the same plane (scale bar = 100  $\mu$ m); 11, tail piece showing that the larger mitochondrial derivative has an associated large deltoid body (large arrow), whereas the smaller mitochondrial derivative has a correspondingly smaller deltoid body (small arrow) (scale bar = 100  $\mu$ m).

identified member of the Diapriidae which belongs to the Proctotrupeoidea *sensu lato* (Quicke *et al.* 1992) and some but not all chalcidoids (Lee and Wilkes 1965; Hogge and King 1975; Quicke 1997). However, the sperm of the only other cynipoid studied to date, *Figites* sp. (Cynipoidea: Figitidae), shows no sign of spiralling (Quicke *et al.* 1992). In all the other hymenopterans with spiral sperm structure, the spiralling includes the axoneme and mitochondrial derivatives as well as the nucleus, and there is no protuberance from the nucleus itself which has a normal circular profile in cross section. The spiralling in wasps other than *Leptopilina* can be better likened to that of an open corkscrew. This phylogenetic distribution of spiralling suggests that this feature is quite homoplastic, but until a robust phylogeny for the Chalcidoidea is available, and more taxa have been investigated spermatologically, it is not possible to say how many times the open corkscrew type spiralling has evolved or been lost. The solid corkscrew form of nucleus with a protruding ridge or keel has not been observed in any other insect, but a quite similar arrangement is found in all Chilopoda, and in the Onychophora and some oligochaetes (Jamieson 1987). In these, the keel is not so well developed as in *Leptopilina* and in some the nucleus itself additionally forms a more or less open corkscrew.

Most of the spermatozoa observed in the sections through the vas deferens were mature, but a few still had a membrane-bound layer of granular cytoplasm surrounding them (Fig. 3) probably indicating that these were still at a late stage of spermiogenesis, implying that not all sperm are mature in the seminal vesicle and vas deferens at least of recently eclosed imaginal wasps.

#### ACKNOWLEDGMENTS

The authors gratefully acknowledge the cheerful assistance of Mr Ian Morris (EM Unit, Department of

Biology, Imperial College). We thank Lex Kraaijeveld for supplying the wasps and Robert Belshaw for comments on an earlier draft. This research was supported by the NERC (Natural Environment Research Council), Initiative in Taxonomy and a grant from the Leverhulme Trust to DLJQ and Mike Fitton (The Natural History Museum).

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